REMARKS

Claims 78, 98-115 and 119-123 are pending in the present application. Claims 1-77, 79-97 and 116-118, have been previously canceled without prejudice or disclaimer. Claims 78, 98-115 and 119-123 have been amended.

Applicants, by canceling or amending any claims, make no admission as to the validity of any rejection made by the Examiner against any such claims. Applicants reserve the right to reassert any of the claims canceled and/or the original claim scope of any claim amended, in a continuing application.

Independent claim 78 is directed to a "method for changing the temperature of a biological matter selected from the group consisting of semen, blood, blood cells, blood constituents and umbilical cord blood from an initial temperature via an intermediate temperature to a final temperature, one of the initial and final temperatures being above the freezing point of the biological matter and the other being below the freezing point, comprising: providing the biological matter in the form of a sample having a minimal dimension in each of two mutually perpendicular cross-sections that exceeds 0.5 centimeters, and at least one of the cross-sections having an outer zone and an inner zone such that the temperature of the sample in the outer zone changes quicker than that in the inner zone; and changing the temperature of the sample, the changing comprising (i) changing the temperature of the sample by subjecting it to a temperature gradient from the initial temperature to the intermediate temperature until the temperature of the sample in at least one part of the outer zone equals the intermediate temperature whilst the temperature of the sample in the inner zone is different from the intermediate temperature, (ii) further

changing the temperature of the sample by subjecting it to the intermediate temperature until the temperature of the sample in at least one cross-section is uniform and equals the intermediate temperature, and (iii) changing the temperature of the sample until the majority of the sample is at the final temperature." Support for this amendment can be found, for example, in the specification at page 4, lines 18 to 21. No new matter has been added.

Claim 121 is directed to a "method for changing the temperature of a biological matter selected from the group consisting of semen, blood, blood cells, blood constituents and umbilical cord blood from an initial temperature via an intermediate temperature to a final temperature, one of the initial and final temperatures being above the freezing point of the matter and the other being below the freezing point, comprising: providing the biological matter in the form of a sample having a minimal dimension in each of two mutually perpendicular cross-sections exceeds 0.5 centimeters, and at least one of the cross-sections having an outer zone and an inner zone such that the temperature of the sample in the outer zone changes quicker than that in the inner zone; and changing the temperature of the sample, the changing comprising (i) (a) changing the temperature of the sample by subjecting it to a temperature gradient from the initial temperature to the intermediate temperature until the temperature of the sample in at least one part of the outer zone equals the intermediate temperature whilst the temperature of the sample in the inner zone is different from the intermediate temperature, the changing is achieved by moving the sample through a region with a temperature gradient from the initial temperature to the intermediate temperature, the sample has a leading end along the direction of movement, (b) moving the leading end of the into a region with a temperature gradient from the initial temperature to the

intermediate temperature, (c) pausing the movement until seeding takes place at the leading end; and moving the sample through the region, (ii) further changing the temperature of the sample by subjecting it to the intermediate temperature until the temperature of the sample in at least one cross-section is uniform and equals the intermediate temperature, and (iii) changing the temperature of the sample until the majority of the sample is at the final temperature, the changing is achieved by moving the sample through a region with a temperature gradient from the intermediate temperature to the final temperature." Support for this amendment can be found, for example, in the specification at page 4, lines 18 to 21. No new matter has been added.

Claim 122 is directed to a "method for changing the temperature of a biological matter selected from the group consisting of semen, blood, blood cells, blood constituents and umbilical cord blood from an initial temperature via an intermediate temperature to a final temperature, one of the initial and final temperatures being above the freezing point of the matter and the other being below the freezing point, comprising: providing the biological matter in the form of a sample having a minimal dimension in each of two mutually perpendicular cross-sections exceeds 0.5 centimeters, and at least one of the cross-sections having an outer zone and an inner zone such that the temperature of the sample in the outer zone changes quicker than that in the inner zone; and changing the temperature of the sample, the changing comprising (i) changing the temperature of the sample by subjecting it to a temperature gradient from the initial temperature to the intermediate temperature until the temperature of the sample in at least one part of the outer zone equals the intermediate temperature whilst the temperature of the sample in the

inner zone is different from the intermediate temperature, (ii) (a) further changing the temperature of the sample by subjecting it to the intermediate temperature until the temperature of the sample in at least one cross-section is uniform and equals the intermediate temperature, the changing is performed by placing the sample in a region with the intermediate temperature, the region having a length along the direction of the movement of the sample and the length is not less the length of the sample along the direction of movement, (b) moving the sample into the region with the intermediate temperature, until substantially the whole sample is within the region, (c) pausing the movement of the sample within the region until the temperature of the sample is substantially uniform throughout the sample and equals the intermediate temperature, (d) moving the sample out of the region, and (iii) changing the temperature of the sample until the majority of the sample is at the final temperature." Support for this amendment can be found, for example, in the specification at page 4, lines 18 to 21. No new matter has been added.

Claim 123 is directed to a "method for changing the temperature of a biological matter selected from the group consisting of semen, blood, blood cells, blood constituents and umbilical cord blood from an initial temperature via an intermediate temperature to a final temperature, one of the initial and final temperatures being above the freezing point of the matter and the other being below the freezing point, comprising: providing the biological matter in the form of a sample having a minimal dimension in each of two mutually perpendicular cross-sections exceeds 0.5 centimeters, and at least one of the cross-sections having an outer zone and an inner zone such that the temperature of the sample

in the outer zone changes quicker than that in the inner zone; and changing the temperature of the sample, the changing comprising (i) changing the temperature of the sample by subjecting it to a temperature gradient from the initial temperature to the intermediate temperature until the temperature of the sample in at least one part of the outer zone equals the intermediate temperature whilst the temperature of the sample in the inner zone is different from the intermediate temperature, (ii) further changing the temperature of the sample by subjecting it to the intermediate temperature until the temperature of the sample in at least one cross-section is uniform and equals the intermediate temperature, and moving the sample into a region with the intermediate temperature and subjecting the sample to the intermediate temperature in the region until the temperature of the sample in each cross-section taken perpendicularly to the direction reaches the intermediate temperature by the time it is moved out of the region, and (iii) changing the temperature of the sample until the majority of the sample is at the final temperature." Support for this amendment can be found, for example, in the specification at page 4, lines 18 to 21. No new matter has been added.

Dependent claims 98-115 and 119 have been amended to be in a form consistent with U.S. practice. No new matter has been added.

In support of the patentability of the presently claimed subject matter, Applicants submit herewith Attachment A. Attachment A describes various experiments conducted according to the presently claimed methods for freezing large volume samples. Additionally, the results were obtained utilizing a system described in the present application. As evidenced by Attachment A: (a) the presently claimed subject matter

permits high recovery of large volumes of cells; (b) the presently claimed subject matter is enabled for cells other than semen, e.g., the additional experiments show high post thawing viability of red blood cells, umbilical cord blood cells and bone marrow cells; (c) the presently claimed methods do not a cryoprotectant in the freezing solution. Should the Examiner require it, Applicants will submit one or more expert declarations substantiating the evidence submitted in Attachment A.

In view of the following, further and favorable consideration is respectfully requested.

I. At page 2 of the Official Action, claims 78, 98-115 and 119-123 have been rejected under 35 USC § 112, first paragraph as failing to comply with the enablement requirement.

The Examiner asserts that the specification does not reasonably provide enablement for the freezing of any biological matter, particularly with no cryoprotectant.

In view of the following, this rejection is respectfully traversed.

From the outset, Applicants note that all of the independent claims, i.e., 78 and 121-123, have been amended to that the biological matter is "selected from the group consisting of semen, blood, blood cells, blood constituents and umbilical cord blood." Accordingly, Applicants submit that the presently claimed subject matter is enabled for the cryopreservation of, at least, semen, blood, blood cells, blood constituents and umbilical cord blood, with or in the absence of a cryoprotectant.

The enablement provision of the Patent Act requires that the patentee provide a written description of the invention "in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly

connected, to make and use the same." 35 U.S.C. § 112, ¶ 1 (2000). The purpose of this requirement is to ensure that "the public knowledge is enriched by the patent specification to a degree at least commensurate with the scope of the claims." *Nat'l Recovery Techs., Inc. v. Magnetic Separation Sys., Inc.*, 166 F.3d 1190, 1195-96 (Fed. Cir. 1999); *see also* Donald S. Chisum, 3 *Chisum on Patents* § 7.01 (2002).

Accordingly, the specification must provide sufficient teaching such that one skilled in the art could make and use the full scope of the invention without undue experimentation. *CFMT, Inc. v. Yieldup Int'l Corp.*, 349 F.3d 1333, 1338 (Fed. Cir. 2003); *Genentech, Inc. v. Novo Nordisk A/S*, 108 F.3d 1361, 1365 (Fed. Cir. 1997); *In re Wands*, 858 F.2d 731, 736-37 (Fed. Cir. 1988). "The key word is 'undue,' not experimentation.'" *Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). Routine experimentation does not constitute undue experimentation. *See Johns Hopkins University v. Cellpro, Inc.*, 152 F.3d 1342 (Fed. Cir. 1998). That is, the specification need only teach those aspects of the invention that one skilled in the art could not figure out without undue experimentation. *See, e.g., Nat'l Recovery Techs.*, 166 F.3d at 1196 ("The scope of enablement . . . is that which is disclosed in the specification plus the scope of what would be known to one of ordinary skill in the art without undue experimentation."); *Wands*, 858 F.2d at 736-37 ("Enablement is not precluded by the necessity for some experimentation such as routine screening."). "Nothing more than objective enablement is required, and therefore it is irrelevant whether this teaching is provided through broad terminology or illustrative examples." *See In re Wright*, 999 F.2d 1557 (Fed. Cir. 1993).

Although the ultimate determination of whether one skilled in the art could make and use the claimed invention without undue experimentation is a legal one, it is based on underlying findings of fact. *CFMT*, 349 F.3d at 1337. Furthermore, "[w]hether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations." *Wands*, 858 F.2d at 737.

Some of these considerations, commonly referred to as "the *Wands* factors," include "(1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims." *Id.; see also Amgen, Inc. v. Chugai Pharm. Co.*, 927 F.2d 1200, 1213 (Fed. Cir. 1991) (stating that the *Wands* factors "are illustrative, not mandatory" and that what is relevant to an enablement determination depends upon the facts of the particular case).

In the present case, Applicants assert that the specification, figures, and examples, provide ample guidance to the skilled artisan in view of the state of the art at the time the application was filed, to make and use the claimed invention without undue experimentation.

Additionally, Applicants note that the court in *In re Wright* held that nothing more than objective enablement is required, and therefore it is irrelevant whether this teaching is provided through broad terminology or illustrative examples.

Applicants submit that reading the present claims in view of the specification a skilled artisan would be able to make and use a method of freezing a biological material

selected from the group consisting of semen, blood, blood cells, blood constituents and umbilical cord blood, with or without a cryoprotectant, as claimed, without undue experimentation.

In support of this position, Applicants submit herewith Attachment A. Attachment A describes various experiments conducted according to the presently claimed methods for freezing large volume samples. Additionally, the results were obtained utilizing a system described in the present application. As evidenced by Attachment A: (a) the presently claimed subject matter permits high recovery of large volumes of cells; (b) the presently claimed subject matter is enabled for cells other than semen, e.g., the additional experiments show high post thawing viability of red blood cells, umbilical cord blood cells and bone marrow cells; (c) the presently claimed methods do not a cryoprotectant in the freezing solution.

With specific reference to Attachment A, Applicants submit that section 1 provides evidence that by utilizing the presently claimed subject matter, red blood cells, frozen as presently claimed, can be thawed with high viability. Specifically, red blood cell samples frozen according to the presently claimed methods, in a freezing vehicle, that, in each of two mutually perpendicular cross-sections, exceeds 0.5 centimeters (the tube having the smallest dimension greater than 0.5 centimeters), viability of 98.72±0.9 of the cells after thawing was achieved. *See* Attachment A at page 1, last paragraph.

Section 2.1 and 2.2 of Attachment A provides evidence that umbilical cord cells frozen according to the presently methods, both with and without a cryoprotectant, have a high viability. More specifically, Applicants note that umbilical cord cells frozen according

to the presently claimed method show high viability, irrespective of the freezing vehicle used. In this regard, when comparing to pre-freezing viability, the viability of the umbilical cord cells described in section 2.1, post thawing, was >100% recovery for the 20ml sample (this is with the known deviation of the counting device, and is taken to represent 100% viability) whereas, for the 1ml samples, there was a 99.4 % and 96.4% recovery. *See* Attachment A at section 2.1. Further, Applicants note that Attachment A, at section 2.2, Table 3, provides evidence that umbilical cord cells frozen without a cryroprotectant also have a high pre and post thawing viability.

Section 3.1 and 3.2 of Attachment A provide evidence that it is possible to obtain high post thawing viability of bone marrow cells frozen according to the presently claimed methods, without a permeating cryoprotectant, e.g., DMSO, in various freezing vehicles. *See* Attachment A at section 3.1 and 3.2.

As discussed in the Response filed on November 6, 2008, page 12, lines 6-30 of the specification as originally filed describe cryropreservation of semen, i.e., a large volume. Additionally, as discussed, at page 13, lines 1-30 and page 14, lines 1-2 of the specification, a cryopreservation in the absence of a cryoprotectant described.

Applicants respectfully submit that both the specification and Attachment A, submitted herewith, provide ample evidence that the specification is enabling for the freezing of biological material selected from the group consisting of semen, blood, blood cells, blood constituents and umbilical cord blood, with or without a cryoprotectant, as claimed.

In view of the foregoing, Applicant submits that the specification enables the skilled artisan to make and use the full scope of claims 78, 98-115 and 119-123, within the meaning of 35 USC § 112, first paragraph. Thus, the Examiner is respectfully requested to withdraw this rejection.

II. At page 5-7 of the Official Action, claims 78, and 98-115 and 119-123 have been rejected under 35 USC § 103 (a) as being unpatentable over US Patent No. 5,873,254 (the '254 patent), both alone and in view of US Patent No. 4,131,200 (the '200 patent).

The Examiner asserts that although the '254 patent does not describe the size of the sample as presently claimed, the generic description in the Summary of the '254 patent is not limited with regard to the size of the sample. With regard to combination of the '254 patent with the '200 patent, the Examiner asserts that the substation of the controlled freezing method described in the '254 patent for the uncontrolled platelet freezing method described in the '200 patent would have been obvious to a person of ordinary skill in the art.

In view of the following, this rejection is respectfully traversed.

To establish a *prima facie* case of obviousness, the PTO must satisfy three requirements. First, as the U.S. Supreme Court very recently held in *KSR International Co. v. Teleflex Inc.*, 550 U.S. 398 (2007), "a court must ask whether the improvement is more than the predictable use of prior art elements according to their established functions. ...it [may] be necessary for a court to look to interrelated teachings of multiple patents; the effects of demands known to the design community or present in the marketplace; and the background knowledge possessed by a person having ordinary skill in the art, all in order to

determine whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue. ...it can be important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does... because inventions in most, if not all, instances rely upon building blocks long since uncovered, and claimed discoveries almost of necessity will be combinations of what, in some sense, is already known." (*KSR*, 550 U.S. 398 at 417.) Second, the proposed modification of the prior art must have had a reasonable expectation of success, determined from the vantage point of the skilled artisan at the time the invention was made. *Amgen Inc. v. Chugai Pharm. Co.*, 18 USPQ2d 1016, 1023 (Fed. Cir. 1991). Lastly, the prior art references must teach or suggest all the limitations of the claims. *In re Wilson*, 165 USPQ 494, 496 (C.C.P.A. 1970).

It is submitted that a proper case of *prima facie* obviousness has not been established because all the elements of the presently claimed subject matter are neither taught nor suggested by the '254 patent and the '200 patent, whether taken alone or in combination. Specifically, Applicants submit that whether taken alone or together, the '254 patent and the '200 patent do not teach or suggest ***a method for changing the temperature of a biological material selected from the group consisting of semen, blood, blood cells, blood constituents and umbilical cord blood having a minimal dimension in each of two mutually perpendicular cross-sections that exceeds 0.5 centimeters***, as presently claimed.

As discussed, claim 78 is directed to a method for changing the temperature of a sample of biological matter selected from the group consisting of semen, blood, blood cells,

blood constituents and umbilical cord blood from an initial temperature via an intermediate temperature to a final temperature, one of the initial and final temperatures being above the freezing point of the biological matter and the other being below the freezing point, comprising: providing the biological matter in the form of a sample having a minimal dimension in each of two mutually perpendicular cross-sections that exceeds 0.5 centimeters, and at least one of the cross-sections having an outer zone and an inner zone such that the temperature of the sample in the outer zone changes quicker than that in the inner zone; and changing the temperature of the sample, the changing comprising (i) changing the temperature of the sample by subjecting it to a temperature gradient from the initial temperature to the intermediate temperature until the temperature of the sample in at least one part of the outer zone equals the intermediate temperature whilst the temperature of the sample in the inner zone is different from the intermediate temperature, (ii) further changing the temperature of the sample by subjecting it to the intermediate temperature until the temperature of the sample in at least one cross-section is uniform and equals the intermediate temperature, and (iii) changing the temperature of the sample until the majority of the sample is at the final temperature. Claims 98-115 and 119 depend, either directly or indirectly, from claim 78.

Claim 121 is directed to a method for changing the temperature of a biological matter selected from the group consisting of semen, blood, blood cells, blood constituents and umbilical cord blood from an initial temperature via an intermediate temperature to a final temperature, one of the initial and final temperatures being above the freezing point of the matter and the other being below the freezing point, comprising: providing the biological

matter in the form of a sample having a minimal dimension in each of two mutually perpendicular cross-sections exceeds 0.5 centimeters, and at least one of the cross-sections having an outer zone and an inner zone such that the temperature of the sample in the outer zone changes quicker than that in the inner zone; and changing the temperature of the sample, the changing comprising (i) (a) changing the temperature of the sample by subjecting it to a temperature gradient from the initial temperature to the intermediate temperature until the temperature of the sample in at least one part of the outer zone equals the intermediate temperature whilst the temperature of the sample in the inner zone is different from the intermediate temperature, the changing is achieved by moving the sample through a region with a temperature gradient from the initial temperature to the intermediate temperature, the sample has a leading end along the direction of movement, (b) moving the leading end of the into a region with a temperature gradient from the initial temperature to the intermediate temperature, (c) pausing the movement until seeding takes place at the leading end; and moving the sample through the region, (ii) further changing the temperature of the sample by subjecting it to the intermediate temperature until the temperature of the sample in at least one cross-section is uniform and equals the intermediate temperature, and (iii) changing the temperature of the sample until the majority of the sample is at the final temperature, the changing is achieved by moving the sample through a region with a temperature gradient from the intermediate temperature to the final temperature.

Claim 122 is directed to a method for changing the temperature of a biological matter selected from the group consisting of semen, blood, blood cells, blood constituents and umbilical cord blood from an initial temperature via an intermediate temperature to a

final temperature, one of the initial and final temperatures being above the freezing point of the matter and the other being below the freezing point, comprising: providing the biological matter in the form of a sample having a minimal dimension in each of two mutually perpendicular cross-sections exceeds 0.5 centimeters, and at least one of the cross-sections having an outer zone and an inner zone such that the temperature of the sample in the outer zone changes quicker than that in the inner zone; and changing the temperature of the sample, the changing comprising (i) changing the temperature of the sample by subjecting it to a temperature gradient from the initial temperature to the intermediate temperature until the temperature of the sample in at least one part of the outer zone equals the intermediate temperature whilst the temperature of the sample in the inner zone is different from the intermediate temperature, (ii) (a) further changing the temperature of the sample by subjecting it to the intermediate temperature until the temperature of the sample in at least one cross-section is uniform and equals the intermediate temperature, the changing is performed by placing the sample in a region with the intermediate temperature, the region having a length along the direction of the movement of the sample and the length is not less the length of the sample along the direction of movement, (b) moving the sample into the region with the intermediate temperature, until substantially the whole sample is within the region, (c) pausing the movement of the sample within the region until the temperature of the sample is substantially uniform throughout the sample and equals the intermediate temperature, (d) moving the sample out of the region, and (iii) changing the temperature of the sample until the majority of the sample is at the final temperature.

Claim 123 is directed to a method for changing the temperature of a biological matter selected from the group consisting of semen, blood, blood cells, blood constituents and umbilical cord blood from an initial temperature via an intermediate temperature to a final temperature, one of the initial and final temperatures being above the freezing point of the matter and the other being below the freezing point, comprising: providing the biological matter in the form of a sample having a minimal dimension in each of two mutually perpendicular cross-sections exceeds 0.5 centimeters, and at least one of the cross-sections having an outer zone and an inner zone such that the temperature of the sample in the outer zone changes quicker than that in the inner zone; and changing the temperature of the sample, the changing comprising (i) changing the temperature of the sample by subjecting it to a temperature gradient from the initial temperature to the intermediate temperature until the temperature of the sample in at least one part of the outer zone equals the intermediate temperature whilst the temperature of the sample in the inner zone is different from the intermediate temperature, (ii) further changing the temperature of the sample by subjecting it to the intermediate temperature until the temperature of the sample in at least one cross-section is uniform and equals the intermediate temperature, and moving the sample into a region with the intermediate temperature and subjecting the sample to the intermediate temperature in the region until the temperature of the sample in each cross-section taken perpendicularly to the direction reaches the intermediate temperature by the time it is moved out of the region, and (iii) changing the temperature of the sample until the majority of the sample is at the final temperature.

Applicants respectfully submit that the presently claimed subject matter is, generally, directed to a method for the cryopreservation of relatively large samples of biological matter selected from the group consisting of semen, blood, blood cells, blood constituents and umbilical cord blood, i.e., ***having a minimal dimension in each of two mutually perpendicular cross-sections exceeding 0.5 centimeters***, and at least one of the cross-sections having an outer zone and an inner zone.

In contrast to the presently claimed subject matter, the '254 patent describes a method for changing the temperature of a sample, whereby the sample is maintained at a second, ***constant*** intermediate temperature, as the sample moves through a thermally conductive block, until the sample's temperature reaches an equilibrium. According to the specific example, and as also admitted by the Examiner, the Examples show that the sample is maintained at the constant intermediate temperature (-7°C) for 10 minutes.

The Examiner asserts that 10 minutes is a sufficient time for the sample to reach an equilibrium. However, Applicants note that ***the questions is not if the time or conditions are sufficient to reach a temperature equilibrium, but rather the amount of damage caused to the cells in the sample during the process***.

The presently claimed subject matter includes subjecting a sample to a second ***temperature gradient*** (beginning at an intermediate temperature) while the sample is conveyed in a tunnel extending through thermally conductive blocks until the temperature of the sample in at least one cross-section is uniform and equals the intermediate temperature. Applicants note that this a moderate process where the sample is gradually subjected to a temperature decrease.

As known by those of ordinary skill in the art, freezing is a heat releasing process. When subjecting a sample containing biological matter to a temperature that is lower than that of sample, at least by several °C, the immediate release of a great amount of heat from the sample causes damage to the matter therein. The greater the temperature difference between the original temperature and the intermediate temperature, the more immediate heat is released and ultimately and the more significant damage is caused to the matter. This damage is avoided by presently claimed methods, where the biological matter is subjected to a temperature gradient while being convened through the thermally conductive blocks.

It is noted that the extent of the damage is also affected by the size of the sample. Large volumes would require longer time until an isotherm is reached. Thus, the combination of large volumes with subjecting the samples to an immediate lower constant temperature, as described by the '254 patent would result in a longer heat release, and thus a greater potential of damaging the cells.

Accordingly, Applicants submit that the freezing method described in the '254 patent is different from the presently claimed methods.

The '200 patent does not remedy the deficiencies of the '254 patent. The '200 patent is directed to a thermoplastic blood bag formed of laminate edge walls enclosing a fluid storage compartment for holding biologically active material such as, blood platelets. However, like the '254 patent, the '200 patent does not teach or suggest the presently claimed cooling methods. Additionally, like the '254 patent, the '200 patent does not teach or suggest a sample that has a minimal dimension exceeding 0.5 cm in each of two

mutually perpendicular cross-sections, as defined for the large volume samples of the present claims. In this regard, Applicants note that a measurement of 9.3X10.2 cm^2 is given in describing Fig. 1, which is a single cross section of the bag. As indicated, "FIG. 1 is an elevation view taken in cross section of a thermoplastic bag of the invention." *See* the '200 patent at column 3, lines 29. However, the '200 patent does not teach or suggest a second cross section being perpendicular to the cross section of Fig. 1 having a minimal dimension larger than 0.5 cm. Therefore, whether taken alone or in combination neither the '254 patent nor the '200 patent teach or suggest ***a method for changing the temperature of a biological material selected from the group consisting of semen, blood, blood cells, blood constituents and umbilical cord blood having a minimal dimension in each of two mutually perpendicular cross-sections that exceeds 0.5 centimeters***, as presently claimed.

In view of the remarks set forth herein, it is submitted that, whether taken alone or in combination, the '200 patent and the '254 patent do not render claims the presently claimed subject matter obvious within the meaning of 35 USC § 103 (a). Accordingly, the Examiner is respectfully requested to withdraw this rejection.

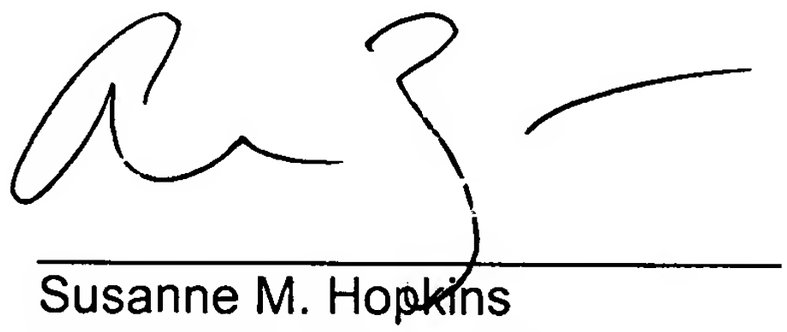
CONCLUSION

In view of the foregoing, Applicant submits that the application is in condition for immediate allowance. Early notice to that effect is earnestly solicited. The Examiner is invited to contact the undersigned attorney if it is believed that such contact will expedite the prosecution of the application.

In the event this paper is not timely filed, Applicants petition for an appropriate extension of time. Please charge any fee deficiency or credit any overpayment to Deposit Account No. 14-0112.

Respectfully submitted,

THE NATH LAW GROUP



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ATTACHMENT A—

SUBMISSION

1. *Red Blood Cells (RBC)*

Packed RBC (pRBC) units were received from the Israeli Blood Services. The pRBC was mixed with a freezing solution (20% (w/v) Dextran 40 (Pharmacosmos, Denmark) and 0.945mg/ml EGCG (Zhejiang Yixin Pharmaceutical, China) both dissolved in saline) at a volume ratio of 1:1 (v/v) solution to blood.

The RBC were mixed with the freezing solution, and 2.5 ml of the thus formed RBC suspension was transferred into glass test tubes (19mm in diameter, this being the smallest dimension of the freezing vehicle). Freezing of the 2.5ml samples was performed according to the presently claimed methods. The first gradient was between 5ºC and -100ºC, with -100°C being the intermediate temperature. Thereafter, the temperature was further lowered to -196°C. The velocity within the blocks was 3mm/sec. The samples were rotated during freezing (60RPM). At the end of the freezing process samples were placed in Liquid nitrogen (LN) storage. Control samples were frozen in a -80°C mechanical freezer. Thawing of the samples was conducted overnight by immersing the samples into a water bath heated to 37ºC.

Recovery (R) was evaluated by measuring supernatant free hemoglobin (f-Hb) according to the cyanmethemoglobin method (Drabkin's reagent, Sigma, USA) and reading absorbance (Abs.) at 540nm with an ELISA reader (EL-800, Bio-Tek Industries, USA).

% Recovery was calculated according to the following equation:

$$\% R = 100 - \% \text{ f-Hb} = 100 - [100 \times \{(\text{Abs. supernatant})/(\text{Abs. supernatant} + \text{Abs. pellet})\}].$$

Recovery was found to be as shown in Table 1:

Table 1: Recovery of RBC after thawing (by free Hb)

	-80ºC	**MTG-516**
Recovery	56.05 ± 5.13	98.72 ± 0.9

Thus, samples frozen according to the presently claimed methods, in a freezing vehicle, that, in each of two mutually perpendicular cross-sections, exceeds 0.5 centimeters (the tube having the smallest dimension greater than 0.5 centimeters), viability of 98.72±0.9 of the cells after thawing was achieved. This viability is much greater than that achieved by conventional freezing at -80°C.

Accordingly, the presently claimed methods are applicable are applicable not only to semen, but any large volume sample, including, e.g., large volumes of RBCs.

2. Umbilical cord blood (UCB)

2.1 A UCB unit after volume reduction (using Hespan) having a volume of 22ml was received from "Sheba" Medical Center. Pure DMSO and Cryocyte (which contains Dextran) were mixed to form a cryopreservation solution at a ratio of 1:1 (v/v) and then 5ml of the cryopreservation solution were added to 20 ml of the volume reduced UCB unit to form a cells suspension.

The cells were counted using an automatic cell counter Pentra 60 (ABX, France) before and after adding the cryopreservation solution, providing $22.1 \cdot 10^6$ cells/ml and $17 \cdot 10^6$ cells/ml, respectively.

One Teflon bag (Permalife PL-120, OriGen Biomedical, USA) was filled with 20ml of the cells suspension and frozen according to the presently claimed methods (V = 0.2mm/sec, T = 0→ -10°C→ -70°C). After freezing, the sample was stored in nitrogen vapor for three days. It is noted that the bag was placed within a frame and the bag with the frame were placed within the system disclosed in the specification, which are configured to performed the presently claimed methods. The combination of the frame with the bag forces the bag to adopt a shape that in each of its two mutually perpendicular cross-sections, exceeds 0.5 centimeters.

In addition two vials (each being about 5.3 cm long with a diameter of about 2.4cm), each containing 1ml of cells suspension prepared as described above, were frozen using a temperature controlled freezer (Mr. Frosty box) which applies a constant rate temperature reduction of 1°C/min. The device was placed at -80°C for 3.5 hours of operation, by the end of which the sample reached -80°C. Thereafter the samples were stored in LN.

Thawing of the bag was achieved by immersing it in a water bath preheated to 37°C. Prior to insertion of the bag into the water bath, the bag was held at room temperature for 60 seconds. Thawing of the bag in the water bath took another 70 seconds (total thawing time of 130 seconds).

The following day the 2 vials were thawed by immersion into a water bath heated to 37°C.

Viability of the UBC cells was determined using live/dead fluorescent stains of Syto/PI.

As shown in Table 2, UCB cells show high viability after being frozen according to the presently claimed methods, irrespective of the freezing vehicle used. Specifically, when comparing to pre-freezing viability, the viability of the cells post thawing was >100% recovery for the 20ml sample (this is with the known deviation of the counting device, and is taken to

represent 100% viability) whereas, for the 1ml samples, there was a 99.4 % and 96.4% recovery.

Table 2: Recovery & Viability of UCB cells post thawing

Sample	Cell concentration ($\cdot 10^6$/ ml)	% Viability
Pre-Freezing	17	90 ± 4.6
Post Thawing bag	17.7	82.6 ± 4.7
Post thawing vial #1	16.9	84 ± 5.3
Post thawing vial #2	16.4	81 ± 5.1

As control, reference is made to the publication by Laroche V et al.[1], which clearly states that there is a loss of 20% recovery of post-thawed cells as compared to pre-freezing.

Thus, the presently claimed methods provide for higher cell viability, as compared to post freezing and thawing using a conventional cell freezing device.

2.2 In a further experiment, recovery of a large volume of UCB cells frozen without a cryopreservation solution was compared to recovery of same cells with a cryopreservation solution, i.e., without a cryoproctant.

Specifically, two units of UCB were separated into blood components using Histopaque-1077 solution. The MNC layer was collected and washed once with PBS (Ca^{++} and Mg^{++} free). The Cell pellet was suspended in a freezing solution (0.1M Trehalose + 0.945mg/ml EGCG, dissolved in PBS).

Two nylon bags, each containing sample volume of 40ml were frozen according to the presently claimed methods (V = 0.2 mm/sec, T= -6°C→ -35°C→ -70°C). It is noted that also in this case the bag is placed within a frame and the bag with the frame are placed within a system configured to performed the presently claimed methods. The combination of the frame with the bag forces the bag to adopt a shape that in each of its two mutually perpendicular cross-sections, exceeds 0.5 centimeters. The two sample containing bags were then stored in -80°C for 4 nights.

Thawing of the samples was conducted by immersing the samples in an Activator thawing device pre-warmed to 37°C. Thawing duration was either 110sec or 90sec. The Activator device is constructed essentially as described in WO2008/032314 (the device shown in Fig. 9A and 9B).

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[1] Laroche V. et al. Cell loss and recovery in umbilical cord blood processing: a comparison of postthaw and postwash samples. Transfusion 45(12):1909-16 (2005), copy enclosed.

Viability of the cells was assayed by Trypan blue exclusion stain. The results are shown in Table 3.

Table 3: Recovery & Viability of UCB cells

Thawing Duration	Pre Freezing Recovery		Post Thawing Recovery	
	Cell con. ($x10^6$/ ml)	% viability (TB*)	Cell con. ($x10^6$/ ml)	% viability (TB*)
110sec	1.6	98.3 ± 3.3	1.5	93.7 ± 8
90sec	2.2	98.6 ± 2.9	1.7	± 8

* The term TB denotes trypan blue exclusion dye, used as known in the art.

The results clearly show that even in the absence of a permeating cryoprotectant, post thawing recovery is very high. Thus, the presently claimed methods are applicable for freezing large volumes of UCB cells without prior mixing with a permeating cryoprotectant.

3. Mice Bone Marrow (MBM) Cells

3.1 MBM cell suspension was prepared from 8 male mice (CB6F1) using RPMI-1640 medium supplemented with 20% FCS and 1% P.S.N and 1% L-glutamine.

The cells were counted using the Pnetra 60 automatic cell counter and the initial cell concentration was $8.8 \cdot 10^6$ cells/ml.

The cell suspension was centrifuged for 10 minutes at 1000g. Cell pellets were re-suspended with two freezing solutions, the 1st, without a permeating cryoprotectant, and the 2nd with a permeating cryoprotectant:

(1) 0.1M Trehalose & 0.945mg/ml EGCG dissolved in PBS (Ca^{++} & Mg^{++} free).

(2) 10% DMSO (v/v) & 10% FCS (v/v) in PBS (Ca^{++} Mg^{++} free)

Four Teflon bags containing 20ml in each (two samples from each freezing solution) were frozen according to the presently claimed methods (V= 0.2 mm/ sec, T = 0°C→ -10°C→-70°C) and stored in LN for 4 days. It is noted that also in this case the bags were placed within a frame and the bag with the frame are placed within a system configured to performed the presently claimed methods. The combination of the frame with the bag forces the bag to adopt a shape that in each of its two mutually perpendicular cross-sections, exceeds 0.5 centimeters.

Thawing was achieved by placing the bags at room temperature (RT) for 90 seconds, after which the bags were placed in a dry thawing device (DTD) essentially as described in WO2008/032314, wherein the bag is sandwiched between two dry metal block having at temperature of 70°C before operation (and is not warmed during operation) for 90 seconds. With respect to the samples containing the 1st freezing solution, prior to freezing, the bags were placed in aluminum bags, due to rupture of the original freezing bags.

Recovery of the cells was evaluated by counting number of cells before and after freezing and thawing. Counting was achieved using the PENTRA-60 automatic cell counter and viability was assayed by Syto/PI (live/dead) fluorescent stains. The results are shown in Table 4 below:

Table 4: Recovery & Viability of MBM

Freezing solution	Pre-freezing		Post thawing		
	Cell con. ($\cdot 10^6$/ ml)	% viability	Cell con. ($\cdot 10^6$/ ml)	% viability	Average
1st solution	2.9	86.1 ±5.6	2.4	58.1 ± 6.2	54 ± 5.8
			2.5	50 ± 7.4	
2nd solution	2.8	91.7 ±4.3	3.1	77.1 ± 6	75.9 ± 1.7
			3.2	74.7 ± 7.3	

The results in Table 4 show that post thawing viabilities were 54% when freezing with a freezing solution free of a permeating cryoprotectant, ***thus supporting the position that by the presently claimed methods the inclusion of a permeating cryoprotectant, such as DMSO, is not mandatory***.

3.2 In a further assay, MBM cells suspension prepared from 8 female mice (Balb C) using RPMI medium with 20% FCS + 1% P.S.N + 1% L-glutamine. The cells were counted ($10.9x10^6$/ ml) and centrifuged (1000g, 10min). Cell pellets were suspended in 160ml a freezing solution (0.1M Trehalose + 0.945 mg/ml EGCG, dissolved in PBS Ca^{++} and Mg^{++} free) to a cell concentration of $2.7x10^6$/ml.

Two test tubes (19mm in diameter, this being the smallest dimension of the freezing vehicle), each containing 2.5ml cell suspension were frozen according to the presently claimed methods (V= 0.2 mm/ sec, T= 5°C→-10°C→-70°C, rpm=60) and stored in LN overnight.

In addition, a 1st nylon bag with tubule containing 80ml and a 2nd nylon bag without the tubule and containing 10ml cell suspension were frozen and

stored in the dry shipper for one night. It is noted that the bag is placed within a frame and the bag with the frame are placed within the system configured to carry out the presently claimed methods (when samples are within a bag). The combination of the frame with the bag forces the bag to adopt a shape that in each of its two mutually perpendicular cross-sections, exceeds 0.5 centimeters.

Thawing of the 1st nylon bag (80ml) was done in the toaster (+70°C) while the thawed cells were poured through the tubule to an Erlenmeyer. Thawing of the 2nd nylon bag (10ml) and the two tubes was done in a water bath (37°C) as described above.

Counting was done by PENTRA and % viability was assayed by Trypan blue. The results of recovery are shown in Table 5 below:

Table 5: Recovery & Viability of MBM

Sample	Pre- Freezing		Post Thawing	
	Cell con. ($x10^6$/ ml)	% viability (TB)	Cell con. ($x10^6$/ ml)	% viability (TB)
80ml bag	2.7	86.4 ± 9	2.4	69 ± 8.1
10ml bag			2.5	79.8 ± 6.6
2.5ml tube (1)			2.6	79.3 ± 7
2.5ml tube (2)			2.5	76.3 ± 9.8

The results presented in Table 5 show that the type of freezing vehicle, and the volume of the sample has no significant effect on the viability of the cells post thawing. ***In other words, the presently claimed methods are applicable not only to semen, but any large volume sample.***